



# Sinocyclocheilus xingrenensis (Cypriniformes, Cyprinidae), a new underground fish from Guizhou Province, Southeastern China

Ming-Yuan Xiao<sup>1\*</sup>, Jia-Jia Wang<sup>2\*</sup>, Tao Luo<sup>3,4</sup>, Jia-Jun Zhou<sup>5</sup>, Ning Xiao<sup>6</sup>, Jiang Zhou<sup>2</sup>

- 1 School of Life Sciences, Guizhou Normal University, Guiyang 550001, Guizhou, China
- 2 School of Karst Science, Guizhou Normal University, Guiyang 550025, Guizhou, China
- 3 School of Life Sciences, Yunnan University, Kunming 650504, Yunnan, China
- 4 Southwest United Graduate School, Kunming 650092, Yunnan, China
- 5 Zhejiang Forest Resource Monitoring Center, Hangzhou 310020, Zhejiang, China
- 6 Guiyang Healthcare Vocational University, Guiyang 550081, Guizhou, China

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Corresponding authors: Ning Xiao (armiger@163.com); Jiang Zhou (zhoujiang@ioz.ac.cn)

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#### Abstract

This study describes a new species, *Sinocyclocheilus xingren*ensis **sp. nov.**, collected from a cave near Xingren City, Guizhou Province, China. Morphologically, the new species can be distinguished from the 80 species currently assigned to the genus *Sinocyclocheilus* by a combination of the following characteristics: absence of horn-like structures and indistinct elevation at the head-dorsal junction; body scaleless, with irregular black markings scattered above the lateral line; tip of pectoral fin not reaching the pelvic fin origin; tip of pelvic fin not reaching the anus; lateral line complete and curved, with pores 65–77; tip of maxillary barbel reaching the anterior margin of the operculum; and six rakers on the first gill arch. Phylogenetic trees constructed based on mitochondrial genes indicate that the new species represents an independent evolutionary lineage with relatively large genetic differences of 2.8–9.0% for the mitochondrial Cyt *b* and 2.7–8.5% for ND4.

# Key Words

Beipanjiang River, Cavefish, morphology, phylogeny, taxonomy

#### Introduction

Southwest China is a region of highly developed karst landscapes, with caves that harbor a wide diversity of cave fishes (Wu et al. 2023). The golden-line fish genus *Sinocyclocheilus* Fang, 1936, a typical taxon of cave fishes endemic to China, is primarily distributed in the karst region of Southwestern China (Zhao and Zhang 2009; Xu et al. 2023) in Guangxi, Guizhou, Yunnan, and Hubei Provinces. After the publication of the type species *S. tingi* of the genus *Sinocyclocheilus* in 1936 (Fang 1936), there was no significant progress in the study of the ge-

nus for decades. However, the study of *Sinocyclocheilus* accelerated after 1980, with many new species being documented based on morphology. More species of *Sinocyclocheilus* were documented after 2010 with the advent of molecular phylogenetics. To date, the genus has been one of the most diversified genera in the family Cyprinidae, comprising more than 80 species (Zhao and Zhang 2009; Jiang et al. 2019; Xu et al. 2023; Luo et al. 2023; Shao et al. 2024; Fan et al. 2024) (Table 1). Since the discovery of the first species in the genus, *Sinocyclocheilus tingi* (Fang 1936), subsequent research demonstrated that due to the uplift of the Qinghai-Xizang Plateau and resulting

<sup>\*</sup> These authors contributed equally to this paper.

climate change (Wen et al. 2022). A recent phylogenetic study, dividing *Sinocyclocheilus* into five species groups, *S. jii*, *S. angularis*, *S. cyphotergous*, *S. microphthalmus*, and *S. tingi* groups (Wen et al. 2022).

As the main species group in the distribution of *Sino*cyclocheilus on the Yunnan-Guizhou Plateau, the S. tingi species group currently includes 27 species (Table 1). These species are mainly found in eastern Yunnan and western Guizhou, including the Nanpanjiang River, Beipanjiang River, Jinshajiang River, Yuanjiang River, and Lancangiang River (Fig. 1). Currently, three species of this species group are recorded in Guizhou Province, i.e., S. angustiporus Zheng & Xie, 1985, S. robustus Chen & Zhao, 1988, and S. xiejiahuai Luo, Fan, Xiao & Zhou, 2024, have been recorded (Fan et al. 2024) (Fig. 1). Moreover, four new species, S. longicornus Luo, Xu, Wu, Zhou & Zhou, 2023; S. xingyiensis Luo, Tang, Deng, Duan & Zhang, 2023; S. guiyang Shao, Chen, Lu, Zhou & Zeng, 2024; and S. xiejiahuai, have recently been described from Guizhou, suggesting that the diversity of the genus in Guizhou may be underestimated.

We collected seven specimens of the genus *Sinocy-clocheilus* during fish surveys in southwestern Guizhou Province between 2012 and 2020. These specimens were morphologically similar to *S. angustiporus*, e.g., having irregular black markings above the lateral line (Wu et al. 1989). However, subsequent morphological comparisons and phylogenetic analyses indicated that these seven specimens represented an undescribed species. Here, we provide the formal description of that new species as *Sinocyclocheilus xingrenensis* sp. nov.

#### Materials and methods

# Specimen sampling, morphological comparison, and statistical analysis

Seven specimens were collected from 2012 to 2020 from several caves in Xingren City, Guizhou Province, China. Gill muscle tissues used for the molecular analysis were preserved in 95% alcohol at -20 °C. All specimens were fixed in 10% buffered formalin and then transferred to 75% ethanol for morphological comparison and long-term preservation. The specimens were deposited at Guizhou Normal University, Guiyang City, Guizhou Province, China.

The new species was placed in the *S. tingi* group based on morphology, e.g., absence of horn-like structures and indistinct elevation at the head-dorsal junction, tip of pectoral fins not reaching the pelvic-fin origin, and the presence of serrations along the posterior margin of the last unbranched fin of the dorsal fin (Zhao and Zhang 2009). Therefore, this study focused on morphological comparisons with the 27 species within the *S. tingi* group (Table 2).

We also examined the type and/or materials from the type-localities of *S. aluensis*, *S. angustiporus*, *S. anophthalmus*, *S. grahami*, *S. huaningensis*, *S. huizeensis*,

S. lateristriatus, S. macrocephalus, S. maitianheensis, S. malacopterus, S. oxycephalus, S. purpureus, S. qi-ubeiensis, S. qujingensis, S. tingi, S. wenshanensis, S. xichouensis, and S. xiejiahuai (Appendix 1).

We measured 34 morphometric characters from a total of seven specimens of the new species referenced from Xu et al. (2023). Principal component analysis (PCA) of corrected morphometric measurements and two-dimensional scatter plots were used to explore the relative contributions of specific variables to the morphological variation. Before the PCA analysis, all measurements were normalized using ratios to standard length (standard length being defined as the ratio to the full length) followed by log transformation. PCA analyses were performed in SPSS 21.0 (SPSS, Inc., Chicago, IL, USA).

# DNA extraction, PCR amplification, and sequencing

We sequenced six samples from the genus Sinocyclocheilus. Genomic DNA was extracted from muscle tissues using a DNA extraction kit (Tiangen Biotech Co., Ltd., Beijing, China). Because the most often used molecular markers for Sinocyclocheilus are fragments of the mitochondrial cytochrome b (Cyt b) and NADH dehydrogenase subunit 4 (ND4) genes, we selected these fragments for amplification and sequencing. The primers used for Cyt b were L14737 (5'-CCAC-CGTTGTTAATTCAACTAC-3') and H15915 (5'-CTC-CGATCTCCGGATTACAAGAC-3'), following Xiao et al. (2005). The primers used for ND4 were L11264 (5'-ACGGGACTGAGCGATTAC-3') and H12346 (5'-TCATCATATTGGGTTAG-3'), following Xiao et al. (2005). PCR amplifications were performed in a 25μl reaction volume with the following cycling conditions: an initial denaturing step at 95 °C for 3 min; 35 cycles of denaturing at 94 °C for 50 s, annealing at 52 °C (for Cyt b and ND4) for 1 min, extension at 72 °C for 1 min, and a final extension step at 72 °C for 10 min. The PCR products were sequenced on an ABI Prism 3730 automated DNA sequencer at Chengdu TSING KE Biological Technology Co., Ltd. (Chengdu, China). All sequences were deposited in GenBank (Table 2).

#### Phylogenetic analyses and genetic distance

We used a total of 116 mitochondrial sequences for molecular analyses, including 61 Cyt *b* sequences and 55 ND4 sequences. Six samples of muscle tissues from *S. guiyang*, *S. aluensis*, *S. angustiporus*, and the new species were sequenced, and 96 sequences were downloaded from GenBank. We selected *Carassius auratus*, *Cyprinus carpio*, *Garra orientalis*, *Neolissochilus hexagonolepis*, *Schizothorax yunnanensis*, *Barbus barbus*, *Onychostoma simum*, *Pethia ticto*, *Myxocyprinus asiaticus*, and *Danio rerio* as outgroups (Wen et al. 2022).

**Table 1.** List of 80 currently recognized species of the genus *Sinocyclocheilus* endemic to China and references. Recognized species modified from Jiang et al. (2019) and Xu et al. (2023).

ID	Species	Species group	Province	Rivers	Literature obtained
${1}$	S. altishoulderus (Li & Lan, 1992)	S. angularis group	Guangxi	Hongshui River	Li and Lan 1992
2	S. anatirostris Lin & Luo, 1986	S. angularis group	Guangxi	Hongshui River	Lin and Luo 1986
3	S. angularis Zheng & Wang, 1990	S. angularis group	Guizhou	Beipanjiang River	Zheng and Wang 1990
4	S. aquihornes Li & Yang, 2007	S. angularis group	Yunnan	Nanpanjiang River	Li et al. 2007
5	S. bicornutus Wang & Liao, 1997	S. angularis group	Guizhou	Beipanjiang River	Wang and Liao 1997
6	S. brevibarbatus Zhao, Lan & Zhang, 2009	S. angularis group	Guangxi	Hongshui River	Zhao et al. 2009
7	S. broadihornes Li & Mao, 2007	S. angularis group	Yunnan	Nanpanjiang River	Li and Mao 2007
8	S. convexiforeheadus Li, Yang & Li, 2017	S. angularis group	Yunnan	Nanpanjiang River	Yang et al. 2017
9	S. hyalinus Chen & Yang, 1994	S. angularis group	Yunnan	Nanpanjiang River	Chen et al. 1994
10	S. Iongicornus Luo, Xu, Wu, Zhou & Zhou, 2023	S. angularis group	Guizhou	Nanpanjiang River	Xu et al. 2023
11	S. jiuxuensis Li & Ran, 2003	S. angularis group	Guangxi	Hongshui River	Li et al. 2003c
12	S. flexuosdorsalis Zhu & Zhu, 2012	S. angularis group	Guangxi	Nanpanjiang River	Zhu and Zhu 2012
13	S. furcodorsalis Chen, Yang & Lan, 1997	S. angularis group	Guangxi	Hongshui River	Chen et al. 1997
14	S. mashanensis Wu, Liao & Li, 2010	S. angularis group	Guangxi	Hongshui River	Wu et al. 2010
15	S. rhinocerous Li & Tao, 1994	S. angularis group	Yunnan	Nanpanjiang River	Li and Tao 1994
16	S. simengensis Li, Wu, Li & Lan, 2018	S. angularis group	Guangxi	Hongshui River	Wu et al. 2018
17	S. tianeensis Li, Xiao & Luo, 2003	S. angularis group	Guangxi	Hongshui River	Li et al. 2003d
18	S. tianlinensis Zhou, Zhang, He & Zhou, 2004	S. angularis group	Guangxi	Nanpanjiang River	Zhou et al. 2004
19	S. tileihornes Mao, Lu & Li, 2003	S. angularis group	Yunnan	Nanpanjiang River	Mao et al. 2003
20	S. xingyiensis Luo, Tang, Deng, Duan & Zhang, 2023	S. angularis group	Guizhou	Nanpanjiang River	Luo et al. 2023
21	S. zhenfengensis Liu, Deng, Ma, Xiao & Zhou, 2018	S. angularis group	Guizhou	Beipanjiang River	Liu et al. 2018b
22	S. anshuiensis Gan, Wu, Wei & Yang, 2013	S. microphthalmus group	Guangxi	Hongshui River	Gan et al. 2013
23	S. microphthalmus Li, 1989	S. microphthalmus group		Hongshui River	Li 1989
24	S. longshanensis Li & Wu, 2018	S. microphthalmus group	Yunnan	Nanpanjiang River	Li et al. 2018
25	S. aluensis Li & Xiao, 2005	S. tingi group	Yunnan	Nanpanjiang River	Li et al. 2005
26	S. angustiporus Zheng & Xie, 1985	S. tingi group	Guizhou; Yunnan	Beipanjiang River; Nanpanjiang River	Zheng and Xie 1985
27	S. anophthalmus Chen & Chu, 1988	S. tingi group	Yunnan	Nanpanjiang River	Chen et al. 1988a
28	S. bannaensis Li, Li & Chen, 2019	S. tingi group	Yunnan	Luosuojiang River	Li et al. 2019
29	S. grahami (Regan, 1904)	S. tingi group	Yunnan	Jinshajiang River	Zhao and Zhang 2009
30	S. guishanensis Li, 2003	S. tingi group	Yunnan	Nanpanjiang River	Li et al. 2003a
31	S. huaningensis Li, 1998	S. tingi group	Yunnan	Nanpanjiang River	Li et al. 1998
32	S. huizeensis Cheng, Pan, Chen, Li, Ma & Yang, 2015	S. tingi group	Yunnan	Niulanjiang River	Cheng et al. 2015
33	S. lateristriatus Li, 1992	S. tingi group	Yunnan	Nanpanjiang River	Li 1992
34	S. longifinus Li, 1998	S. tingi group	Yunnan	Nanpanjiang River	Li et al. 1998
35	S. macrocephalus Li, 1985	S. tingi group	Yunnan	Nanpanjiang River	Li 1985
36	S. maculatus Li, 2000	S. tingi group	Yunnan	Nanpanjiang River	Li et al. 2000a
37	S. maitianheensis Li, 1992	S. tingi group	Yunnan	Nanpanjiang River	Li 1992
38	S. malacopterus Chu & Cui, 1985	S. tingi group	Yunnan	Nanpanjiang River	Chu and Cui 1985
39	S. oxycephalus Li, 1985	S. tingi group	Yunnan	Nanpanjiang River	Li 1985
40	S. purpureus Li, 1985	S. tingi group	Yunnan	Nanpanjiang River	Li 1985
41	S. qiubeiensis Li, 2002	S. tingi group	Yunnan	Nanpanjiang River	Li et al. 2002b
42	S. qujingensis Li, Mao & Lu, 2002	S. tingi group	Yunnan	Nanpanjiang River	Li et al. 2002c
43	S. robustus Chen & Zhao, 1988	S. tingi group	Guizhou	Nanpanjiang River	Chen et al. 1988b
44	S. tingi Fang, 1936	S. tingi group	Yunnan	Nanpanjiang River	Zhao and Zhang 2009
45	S. wenshanensis Li, Yang, Li & Chen, 2018	S. tingi group	Yunnan	Jinshajiang River	Yang et al. 2018
46	S. wumengshanensis Li, Mao, Lu & Yan, 2003	S. tingi group	Yunnan	Jinshajiang River	Li et al. 2003a
47	S. xichouensis Pan, Li, Yang & Chen, 2013	S. tingi group	Yunnan	Panlonghe River	Pan et al. 2013
48	S. yangzongensis Chu & Chen, 1977	S. tingi group	Yunnan	Nanpanjiang River	Zhao and Zhang 2009
49	S. yimenensis Li & Xiao, 2005	S. tingi group	Yunnan	Yuanjiang River	Li et al. 2005

ID	Species	Species group	Province	Rivers	Literature obtained
50	S. xiejiahuai Fan, Luo, Xiao & Zhou, 2024	S. tingi group	Guizhou	Beipanjiang River	Fan et al. 2024
51	S. macroscalus Li, 1992	S. tingi group	Yunnan	Nanpanjiang River	Li 1992
52	S. brevis Lan & Chen, 1992	S. cyphotergous group	Guangxi	Liujiang River	Chen and Lan 1992
53	S. cyphotergous (Dai, 1988)	S. cyphotergous group	Guizhou	Hongshui River	Huang et al. 2017
54	S. donglanensis Zhao, Watanabe & Zhang, 2006	S. cyphotergous group	Guangxi	Hongshui River	Zhao et al. 2006
55	S. dongtangensis Zhou, Liu & Wang, 2011	S. cyphotergous group	Guizhou	Liujiang River	Zhou et al. 2011
56	S. gracilicaudatus Zhao & Zhang, 2014	S. cyphotergous group	Guangxi	Liujiang River	Wang et al. 2014
57	S. huanjiangensis Wu, Gan & Li, 2010	S. cyphotergous group	Guangxi	Liujiang River	Wu et al. 2010
58	S. hugeibarbus Li, Ran & Chen, 2003	S. cyphotergous group	Guizhou	Liujiang River	Li et al. 2003b
59	S. lingyunensis Li, Xiao & Lu, 2000	S. cyphotergous group	Guangxi	Hongshui River	Li et al. 2000b
60	S. longibarbatus Wang & Chen, 1989	S. cyphotergous group	Guizhou; Guangxi	Liujiang River	Wang and Chen 1989
61	S. luopingensis Li & Tao, 2002	S. cyphotergous group	Yunnan	Nanpanjiang River	Li et al. 2002a
62	S. macrolepis Wang & Chen, 1989	S. cyphotergous group	Guizhou; Guangxi	Liujiang River	Wang and Chen 1989
63	S. macrophthalmus Zhang & Zhao, 2001	S. cyphotergous group	Guangxi	Hongshui River	Zhang and Zhao 2001
64	S. multipunctatus (Pellegrin, 1931)	S. cyphotergous group	Guizhou; Guangxi	Hongshui River; Wujiang River	Zhao and Zhang 2009
65	S. punctatus Lan & Yang, 2017	S. cyphotergous group	Guizhou; Guangxi	Liujiang River; Hongshui River	Lan et al. 2017
66	S. ronganensis Luo, Huang & Wen, 2016	S. cyphotergous group	Guangxi	Liujiang River	Luo et al. 2016
67	S. sanxiaensis Jiang, Li, Yang & Chang, 2019	S. cyphotergous group	Hubei	Yangtze River	Jiang et al. 2019
68	S. xunlensis Lan, Zhan & Zhang, 2004	S. cyphotergous group	Guangxi	Liujiang River	Lan et al. 2004
69	S. yaolanensis Zhou, Li & Hou, 2009	S. cyphotergous group	Guizhou	Liujiang River	Zhou et al. 2009
70	S. yishanensis Li & Lan, 1992	S. cyphotergous group	Guangxi	Liujiang River	Li and Lan 1992
71	S.guiyang Shao, Chen, Lu, Zhou, Zeng, 2024	S. cyphotergous group	Guizhou	Wujiang River	Shao et al. 2024
72	S. brevifinus Li, Li & Mayden, 2014	S. jii group	Guangxi	Hejiang River	Li et al. 2014
73	S. guanyangensis Chen, Peng & Zhang, 2016	S. jii group	Guangxi	Guijiang River	Chen et al. 2016
74	S. guilinensis Ji, 1985	S. jii group	Guangxi	Guijiang River	Zhao and Zhang 2009
75	S. huangtianensis Zhu, Zhu & Lan, 2011	S. jii group	Guangxi	Hejiang River	Zhu et al. 2011
76	S. jii Zhang & Dai, 1992	S. jii group	Guangxi	Hejiang River	Zhang and Dai 1992
77	S. gracilis Li, 2014	No assignment	Guangxi	Guijiang River	Li and Li 2014
78	S. luolouensis Lan, 2013	No assignment	Guangxi	Hongshui River	Lan et al. 2013
79	S. pingshanensis Li, Li, Lan & Wu, 2018	No assignment	Guangxi	Liujiang River	Wu et al. 2018
80	S. wui Li & An, 2013	No assignment	Yunnan	Mingyihe River	Li and An 2013

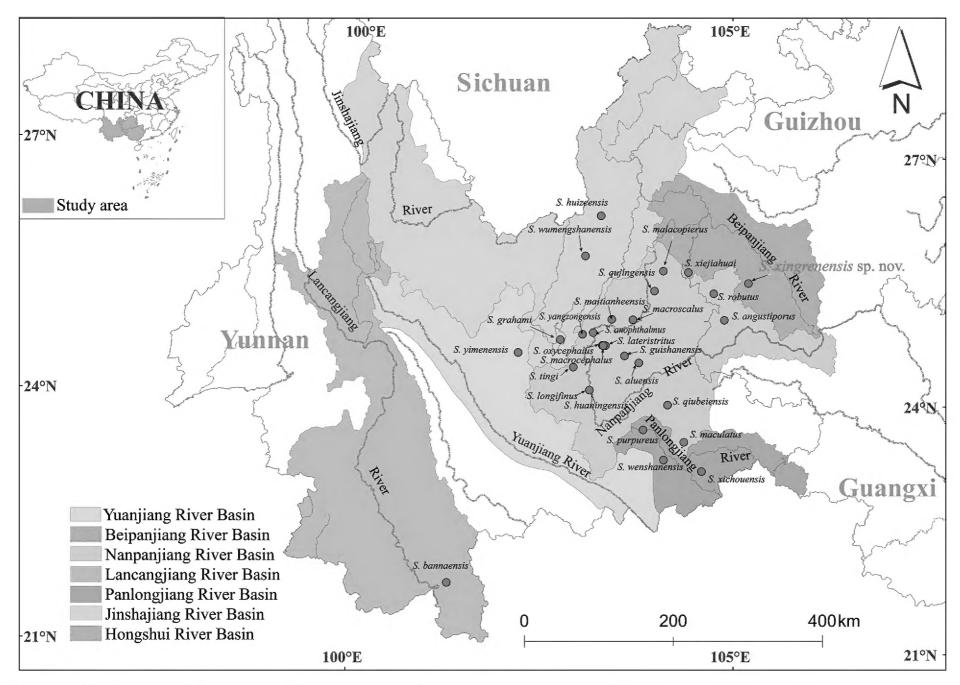
The sequences were revised manually and then aligned using the MUSCLE (Edgar 2004) module in MEGA v.7.0 (Kumar et al. 2016) with default settings. Phylogenetic trees were constructed using both maximum likelihood (ML) and Bayesian inference (BI) methods. The best-fit partitioning scheme and nucleotide substitution models for the sequence matrix were selected within Partition-Finder v.2.1.1 (Lanfear et al. 2017) based on the Bayesian information criterion. The analysis suggested the best partition scheme for each codon position of the Cyt b and ND4 genes. Maximum Likelihood analysis was run in IQ-TREE v.1.6.1 (Nguyen et al. 2015) with the best model and 2000 ultrafast bootstrap (UBP) replicates. The BI tree was reconstructed using MrBayes v.3.2.1 (Ronquist et al. 2012). Two independent runs were conducted in the BI analysis, each of which was performed for 10 million generations and sampled every 1000 generations. The first 25% of the run samples were discarded as a burn-in. Convergence of the data runs was assessed using the average

standard deviation of split frequencies (below 0.01) and checking effective sample size (more than 200) in Tracer v1.7.1 (Rambaut et al. 2018). Uncorrected *p*-distances (1000 replicates) based on the Cyt *b* and ND4 genes were calculated using MEGA v 7.0 (Kumar et al. 2016).

## Results

# Phylogenetic reconstruction and genetic divergence

The ML and BI phylogenies were constructed based on two concatenated mitochondrial sequences comprising 1140 bp of Cyt *b* and 1380 bp of ND4. As a result of model selection, GTR+I+G and HKY+I+G were selected as the best models for the 1<sup>st</sup> codon and 2<sup>nd</sup> codon of ND4 and Cyt *b*, as well as GTR+I+G and GTR+G as the best models for the 3<sup>rd</sup> codon of ND4 and Cyt *b*. The ML



**Figure 1.** Distribution of 27 species of *S. tingi* group and new species in southwest China.

and BI phylogenetic trees showed identical topologies (Fig. 2). The monophyly of the genus *Sinocyclocheilus* was strongly supported by both phylogenetic analyses. In both analyses, *S. xingrenensis* sp. nov. formed a highly supported clade (BPP/UBP = 0.78/92) (Fig. 2).

We calculated the genetic distance between the new species and the *S. tingi* group using the mitochondrial Cyt *b* and ND4 genes. The smallest *p*-distances between *S. xingrenensis* sp. nov. and other species within the group were 2.8% for Cyt *b* (vs. *S. macrocephalus* and *S. aluensis*) and 2.7% for ND4 (vs. *S. macrocephalus*). These levels of divergence were similar to those between pairs of other recognized species. For example, the Cyt *b p*-distance was 2.6% between *S. aluensis* and *S. huaningensis* and 2.7% between *S. guishanensis* and *S. macrocephalus*, while the ND4 *p*-distance was 2.0% between *S. guishanensis* and *S. macrocephalus*, while the ND4 *p*-distance was 2.0% between *S. guishanensis* and *S. macrocephalus* (Suppl. materials 1, 2).

#### Morphological analyses

A total of five principal component factors with eigenvalues greater than one were extracted based on the morphometric data (Suppl. material 3). These accounted for 91.72% of the total variance, with the first principal component (PC1) and second principal component (PC2) accounting for 60.20% and 15.10% of the total variance,

respectively. In the scatter plot of PC1 versus PC2, the new species *Sinocyclocheilus xingrenensis* sp. nov. was distinguishable from *S. angustiporus* and *S. robustus* on the PC1 axis (Fig. 3). Major morphometric characters loaded on the PC1 axis included body depth, dorsal fin length, preanal length, anal-fin base length, anal-fin depth, prepectoral length, caudal peduncle length, head depth, head width, eye diameter, upper jaw length, lower jaw length, mouth width, and rostral barbel length (Suppl. material 3). Multivariate analysis of variance showed that, except for body depth, *Sinocyclocheilus xingrenensis* sp. nov. was greater than *S. angustiporus* in the remaining 27 morphometric indices in general, and the differences were significant (Table 4).

#### Morphological comparisons

Based on phylogeny and morphology, the new species was classified into the *S. tingi* group, inclding indistinct elevation at the head-dorsal junction, last unbranched ray of dorsal fin serrate along posterior margin, absence of horn-like structures and indistinct elevation at the head-dorsal junction, and tip of pectoral fin not reaching the pelvic-fin origin (Zhao and Zhang 2009). Thus, the new species *Sinocyclocheilus xingrenensis* sp. nov. were compared in detail morphologically with literature data for the *S. tingi* group (Table 3).

Table 2. Localities, voucher information, and GenBank numbers for all samples used.

ID	Species	Locality (* type localities)	Voucher number	Cyt b	ND4
1	S. xingrenensis sp. nov.	Xingren City, Guizhou*	XR01	PQ893907	PQ893904
2	S. xingrenensis sp. nov.	Xingren City, Guizhou*	XR02	PV007875	NA
3	S. xingrenensis sp. nov.	Xingren City, Guizhou*	XR03	PV007876	NA
4	S. altishoulderus	Mashan County, Guangxi	NA	FJ984568	FJ984568
5	S. aluensis	NA	NA	PV007877	NA
6	S. anatirostris	Leye County, Guangxi	XH1901	AY854708	AY854765
7	S. angularis	Baotian Town, Panzhou City, Guizhou*	GZNU20210322001	MZ636514	MZ636514
8	S. angustiporus	Xinlongchnag Town, Xingren City, Guizhou	GZNU20210322002	MZ636515	MZ636515
9	S. angustiporus	Yunnan	NA	PV007878	NA
10	S. anophthalmus	Jiuxiang, Yiliang County, Yunnan	XH3001	AY854715	AY854772
11	S. anshuiensis	Lingyun County, Guangxi	NA	KR069120	KR069120
12	S. bicornutus	Xinlongchang Town, Xingren City, Guizhou*	NA	KX528071	KX528071
13	S. brevibarbatus	NA	GX0064-L20-13	MT373106	MW548423
14	S. brevis	NA	GX0155	MT373105	MW548424
15	S. cyphotergous	Dongdang Township, Luodian County, Guizhou*	GZNU20150819010	MW024370	MW024370
16	S. donglanensis	Hongshuihe River, Donglan County, Guangxi	CA139	AB196440	MW548425
17	S. furcodorsalis	Tian'e County, Guangxi	NA	GU589570	GU589570
18	S. grahami	Haikou, Kunming City, Yunnan	NA	GQ148557	GQ148557
19	S. guanyangensis	NA	GX0173	MT373108	MW548426
20	S. guilinensis	NA	GX0073-L17-2	MT373104	MW548427
21	S. guishanensis	Guishan, Shilin County, Yunnan	XH5401	AY854722	AY854779
22	S. guiyang	Guiyang City, Guizhou, China*	NA	PV007879	PV007880
23	S. huangtianensis	NA	GX0175	MT373109	MW548428
24	S. huaningensis	Huaning County, Yunnan	XH3701	AY854718	AY854775
25	S. huanjiangensis	NA	GX0124	MT373103	MW548429
26	S. hugeibarbus	Xiaoqikong Town, Libo County, Guizhou*	GZNU20150120005	MW014319	MW014319
27	S. huizeensis	Leye Town, Huize County, Yunnan	hrfri2018046	MH982229	MH982229
28	S. hyalinus	Alugudong, Luxi County, Yunnan	XH4701	AY854721	AY854778
29	S. jii	Gongcheng County, Guangxi	YNUSJ201308060038	MF100765	MF100765
30	S. jiuxuensis	Jiuxu Town, Hechi City, Guangxi	XH8501	AY854736	AY854793
31	S. lateristriatus	Maojiachong, Zhanyi County, Yunnan	XH1102	AY854703	AY854760
32	S. lingyunensis	Shadong, Lingyun County, Guangxi	XH0502	AY854691	AY854748
33	S. longibarbatus	Dongtang Township, Libo County, Guizhou*	GZNU20150809004	MW024372	MW024372
34	S. longicornus	Hongguo Town, Panzhou City, Guizhou*	GZNU20210503016	MZ634123	MZ634125
35	S. lunanensis	Shilin County, Yunnan	XH0302	AY854686	AY854743
36	S. macrocephalus	Heilongtan, Shilin County, Yunnan	XH0103	AY854683	AY854740
37	S. macrolepis	Nandan County, Guangxi	XH8201	AY854729	AY854786
38	S. macrophthalmus	Xiaao, Duan County, Guangxi	XH8401	AY854733	AY854790
39	S. maculatus	Yiliang, Yunnan	IHB:2006632	EU366193	EU366183
40	S. maitianheensis	Jiuxiang, Yiliang County, Yunnan	XH2301	AY854710	AY854767
41	S. malacopterus	Wulonghe, Shizong County, Yunnan	XH0901	AY854697	AY854754
42	S. mashanensis	NA NA	GX0026-L18-12	MT373107	MW548430
43	S. microphthalmus	Lingyun County, Guangxi	NNNU201712001	MN145877	MN145877
44	S. multipunctatus	Huishui County, Guizhou	NA	MG026730	MG026730
45	S. oxycephalus	Heilongtan, Shilin County, Yunnan	XH0201	AY854685	AY854742
46	S. punctatus	Dongtang Township, Libo County, Guizhou	GZNU20150811002	NC_058003	NC_058003
47	S. purpureus	Luoping County, Yunnan	IHB:2006638	EU366189	EU366178
48	S. qiubeiensis	Songming, Yunnan	IHB:2006624	EU366188	EU366182
49	S. qujingensis	Huize County, Yunnan	hrfri2018044	MH937706	MH937706
. 0	- 1.9	Journey, Furnium			

ID	Species	Locality (* type localities)	Voucher number	Cyt b	ND4
50	S. rhinocerous	Luoping County, Yunnan	NA	KR069119	KR069119
51	S. ronganensis	Rong'an County, Guangxi	NA	KX778473	KX778473
52	S. sanxiaensis	Guojiaba Town, Zigui County, Hubei*	KNHM 2019000001	MN106258	NA
53	S. tianlinensis	NA	GX0087-L17-16	MT373102	MW548431
54	S. tingi	Fuxian Lake, Yunnan	YNUST201406180002	MG323567	MG323567
55	S. wumengshanensis	Xuanwei County, Yunnan	YNUSM20160817008	MG021442	MG021442
56	S. xiejiahuai	Hongguo Town, Panzhou City, Guizhou*	S46	PQ165088	PQ165088
57	S. xunlensis	Huanjiang, Guangxi	IHB:04050268	EU366187	EU366184
58	S. yangzongensis	Yangzonghai Lake, Yunnan	XH6101	AY854725	AY854782
59	S. yimenensis	Yimen, Yunnan	IHB:2006646	EU366191	EU366180
60	S. yishanensis	Liujiang County, Guangxi	NA	MK387704	MK387704
61	S. zhenfengensis	Zhexiang Town, Zhenfeng County, Guizhou*	GZNU20150112021	MW014317	MW014317
62	Carassius auratus	NA	NA	AB111951	AB111951
63	Cyprinus carpio	NA	NA	JN105357	JN105357
64	Garra orientalis	NA	NA	JX290078	JX290078
65	Neolissochilus hexagonolepis	NA	NA	KU380329	KU380329
66	Schizothorax yunnanensis	NA	NA	KR780749	KR780749
67	Barbus barbus	NA	NA	AB238965	AB238965
68	Onychostoma simum	NA	NA	KF021233	KF021233
69	Pethia ticto	NA	NA	AB238969	AB238969
70	Myxocyprinus asiaticus	NA	NA	AY526869	AY526869
71	Danio rerio	NA	NA	KM244705	KM244705

Sinocyclocheilus xingrenensis sp. nov. can be distinguished from the 21 species belonging to the *S. angularis* group by the absence of horn-like structures and indistinct elevation at the head-dorsal junction (vs. presence) and tip of pectoral fins not reaching the pelvic-fin origin (vs. reaching the or beyond the pelvic-fin origin); from the three species in the *S. microphthalmus* group by an indistinct elevation at the head-dorsal junction (vs. distinct elevation); from the five species within the *S. jii* group with serrations along the posterior margin of the last unbranched fin of the dorsal fin (vs. absent); and from the 20 species in the *S. cyphotergous* group by an indistinct elevation at the head-dorsal junction (vs. distinct elevation) and tip of pectoral fins not reaching the pelvic-fin origin (vs. usually reaching the pelvic-fin origin) (Zhao and Zhang 2009).

For the 27 species of the *S. tingi* group, the new species can be distinguished by a series of morphological characters. By having irregular markings on the body lateral, the new species can be distinguished from *S. anophthalmus*, *S. longifinus*, *S. macrocephalus*, *S. qujingensis*, *S. xiejiahuai*, and *S. yangzongensis* (vs. presence). *Sinocyclocheilus xingrenensis* sp. nov. differs from *S. aluensis*, *S. bannaensis*, *S. grahami*, *S. guishanensis*, *S. huaningensis*, *S. huizeensis*, *S. lateristriatus*, *S. maitianheensis*, *S. malacopterus*, *S. macroscalus*, *S. purpureus*, *S. qiubeiensis*, *S. tingi*, *S. wenshanensis*, *S. wumengshanensis*, *S. xichouensis*, and *S. yimenensis* by body scaleless (vs. body covered with scales); from *S. maculatus* by lateral-line pores 65–76 (vs. 81–88), six gill rakers on first gill arch (vs. 14–17), irregular black markings mainly scattered above

the lateral line (vs. irregular markings densely distributed on the body lateral) (Zhao and Zhang 2009); and from *S. oxycephalus* by body length 4.4–5.0 times body height (vs. 3.3–4.1 times), head length greater than body height (vs. head length equal to body height), head length 3.4–3.9 times mouth width (vs. 4.3–5.1 times), and tip of maxillary barbel reaching the anterior margin of the operculum (vs. reaching the posterior margin of the operculum) (Li 1985; Zhao and Zhang 2009).

The new species are morphologically close to S. angustiporus and S. robustus and were found to remain easily distinguishable by examining their type specimens. The new species can distinguish S. angustiporus by three unbranched dorsal-fin rays (vs. four), body scaleless (vs. body covered with tiny scales), six rakers on the first gill arch (vs. 7–9), tip of maxillary barbel reaching the anterior margin of the operculum (vs. reaching the posterior margin of the eye), larger body size  $(92.6 \pm 15.0 \text{ mm vs. } 59.2 \text{ mm})$  $\pm 25.5$  mm, p = 0.017), bigger eyes  $(4.7 \pm 0.3$  mm vs.  $3.7 \pm 0.3$ 0.9 mm, p = 0.020), longer head (25.0 ± 3.1 mm vs. 15.8 ± 6.4 mm, p = 0.008), and longer fins (all p-values were less than 0.05) (Table 4), and it is distributed in the Beipanjiang River basin (vs. the Nanpanjiang River basin). The new species can distinguish S. robustus by smaller body size (standard length 92.6 mm vs. standard length 162.7), three unbranched dorsal-fin rays (vs. four), eight branched pelvic-fin rays (vs. six), six rakers on the first gill arch (vs. 9), indistinct elevation at the head-dorsal junction (vs. distinct elevation) (Figs 4, 6), dorsal-fin origin opposite to the pelvic-fin origin (vs. dorsal-fin origin anterior to the



**Figure 2.** Phylogenetic tree based on mitochondrial Cyt b + ND4 genes. In this phylogenetic tree, ultra-fast bootstrap supports (UBP) from ML analyses/Bayesian posterior probabilities (BPP) from BI analyses were noted beside nodes. The scale bar represents 0.07 nucleotide substitutions per site. In addition to the nodes, different-colored rectangles indicate the presence or absence of scales for species in the genus *Sinocyclocheilus*.

pelvic-fin origin), and distributed in the Beipanjiang river basin (vs. Nanpanjiang river basin).

For the four species not placed in any species group, the new species differs from *S. pingshanensis*, *S. gracilis*, and *S. wui* by having irregular markings on the lateral body and body scaleless (vs. lacking irregular markings and body covered with tiny scales). The new species differs from *S. luolouensis* by eye normal (vs. eyes reduced), body scaleless (vs. body covered with tiny scales), lateral-line pores 65–77 (vs. 40–49), and tip of pectoral fin not reaching the pelvic-fin origin (vs. reaching the pelvic-fin origin).

#### Taxonomic account

Sinocyclocheilus xingrenensis Luo, Xiao, Zhou, Xiao & Zhou, sp. nov.

https://zoobank.org/53D64357-99FA-4E10-9590-002AFA9A39EE Fig. 4, Table 4

**Chresonymy.** *Sinocyclocheilus gaowuensis*: Liu 2018 (Xingren City, Guizhou Province, China).

**Material examined.** *Holotype.* • GZNU20190508001, total length 149.4 mm (TL), standard length 123.5 mm (SL), collected by Jia-Jun-Zhou on May 8, 2019, in Yangsitun Vil-

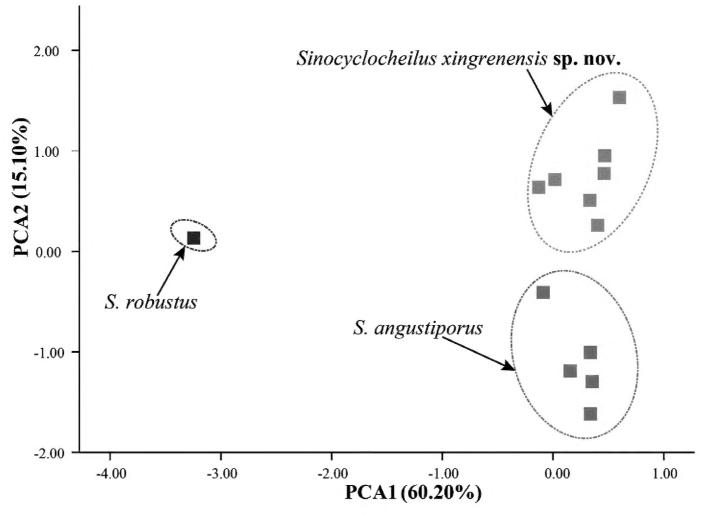


Figure 3. Scatter plots of the 1st and 2nd principal components for S. robustus, S. angustiporus, and Sinocyclocheilus xingrenensis sp. nov.

lage, Tianba Community, Xingren City, Guizhou Province, China (25.41255686°N, 105.21536082°E; ca. 1323 m a.s.l.).

*Paratypes.* • Six specimens from the same locality as the holotype: GZNU20190508002–GZNU20190508007, 79.7–123.5 mm SL, collected by Jia-Jun-Zhou on May 8, 2019; GZNU201250907001, 69.5 mm SL, collected by Tao Liu on September 7, 2012, in Gaowu Village, Xiashan Town, Xingren City, Guizhou Province, China (25.52536100°N, 105.20758400°E; ca. 1415 m a.s.l.).

**Diagnosis.** Sinocyclocheilus xingrenensis sp. nov. can be distinguished from its congeners by a combination of the following characteristics: (1) absence of horn-like structures and indistinct elevation at the head-dorsal junction; (2) body scaleless, with irregular black markings scattered above the lateral line; (3) eyes large, diameter 16–21% of head length; (4) dorsal-fin rays, iii, 6–7, last unbranched ray serrate along posterior margin; (5) tip of the pectoral fin not reaching the pelvic fin origin; (6) pelvic fin rays, i, 8, tip not reaching the anus; (7) lateral line complete and curved, with pores 65–77; (8) tip of maxillary barbel reaching the anterior margin of the operculum; (9) six rakers on the first gill arch. The major diagnostic characters for new species and related species are summarized in Table 3.

**Description.** Body fusiform, moderately elongated and compressed. Dorsal profile convex from nape to dorsal fin; body maximum depth positioned at insertion of dorsal fin; ventral profile slightly concave, tapering gradually toward the caudal fin.

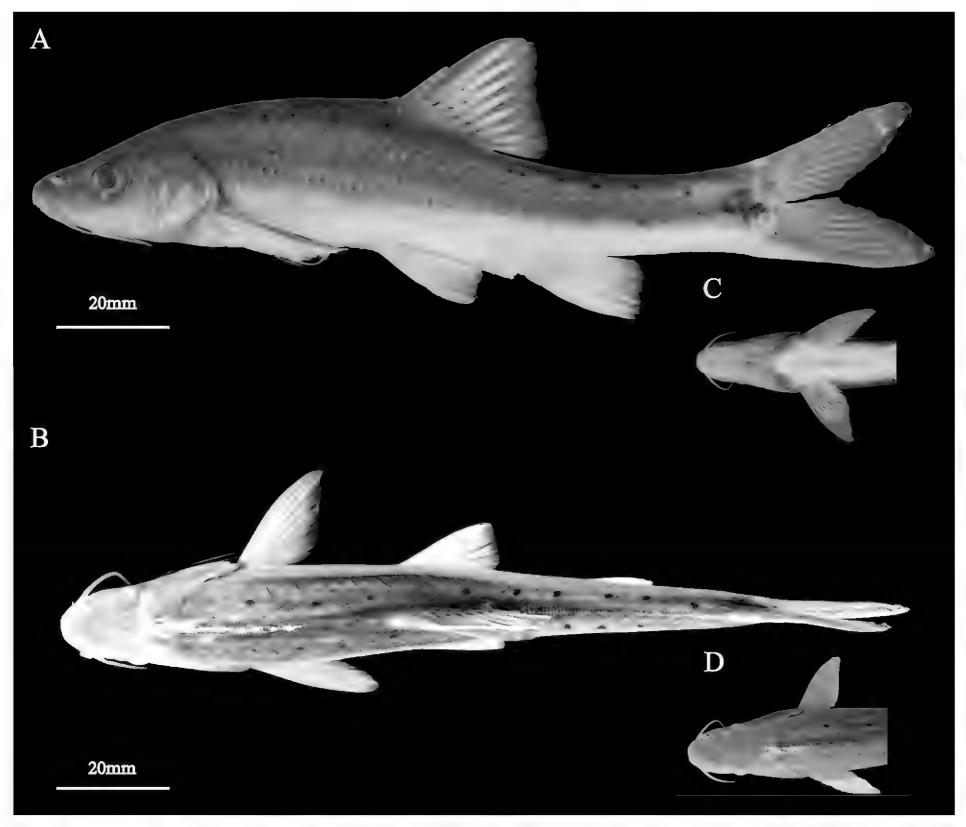
Head short, length, 26–29% of SL, slightly compressed, head length greater than width. Eyes large, eye diameter 16–21% of HL, interorbital distance greater than distance between posterior nostrils. Snout short,

U-shaped, and projecting beyond lower jaw in dorsal view, less than half HL. Mouth subinferior and arched, with slightly projecting lower jaw. Two pairs of nostrils, anterior nostril close to posterior nostril, nares at about 1/3 between snout tip and anterior margin of eye; anterior nostril short tubular, posterior margin of short tube with posterior flap, forward to cover mouth of tube; posterior nostril subcircular, open. Two pairs of barbels; rostral barbels not reaching the anterior margin of operculum when extended backward; maxillary barbel slightly shorter compared with rostral barbel, tips beyond the eye but reaching anterior margin of operculum when extended backward. Gill opening moderate, opercular membranes connected at isthmus. Six outer rakers on first gill arch. Pharyngeal teeth in three rows with counts of 2, 3, 4–4, 3, 2; pharyngeal teeth strong and well developed, with curved and pointed tips.

Dorsal-fin rays iii, 6–7; pectoral-fin rays i, 12–16; pelvic-fin rays i, 8; anal-fin rays iii, 5; and 15–18 branched caudal-fin rays. Dorsal fin short, 19–24% of SL, less than head length, 67–88% of HL; distal margin truncated, origin opposite to pelvic-fin origin, situated slightly anterior to midpoint between snout tip and caudal-fin base; last unbranched ray strong, softening toward tip, with serrations along posterior margin; first branched ray longest, shorter than HL, tip beyond the vertical of the anus. Pectoral fin short, distal margin truncated, length slightly smaller than HL, 18–22% of SL, tips beyond 3/4 of the distance between pectoral-fin origin and pelvic-fin origin, tips not reaching the pelvic-fin origin. Pelvic fin moderately developed, distal margin rounded, length 14–17% of SL, and tips not reaching the anus. Anal fin short, 14–17% of SL, distal margin rounded, origin close to the anus, a short

**Table 3.** Comparison of the diagnostic characters of the new species described here with those selected for the 27 species of the *S. tingi* group and the four unassigned species (the last four) within the genus *Sinocyclocheilus*. Grey shading indicates a clear difference in characters compared to that of *Sinocyclocheilus xingrenensis* sp. nov.

Species	Body lateral markings	Dorsal-fin rays	Pectoral-fin rays	Anal-fin rays	Pelvic-fin rays	Caudal -fin rays	Lateral-line scales <sub>/</sub> pores	Body scales	Tip of pectoral fin reaching to pelvic-fin	Tip of pelvic-fin rays reaching to anus	Source
S. xingrenensis sp.	Present	iii, 6–7	i, 12–16	iii, 5	i, 8	15–18	65–77	No	No	No	This study
S. aluensis	Present	iii, 7	i, 13–16	ii, 5	i, 7–9	15–17	71–75	Yes	No	No	Li et al. 2005
S. angustiporus	Present	iv, 7	i, 14	iii, 5	i, 8	16–17	70–74	Yes	No	No	Wu et al. 1989; This study
S. anophthalmus	Absent	iv, 8	i, 15–16	iii, 5	i, 8	16	52–56	Yes	Yes	No	Chen et al. 1988a
S. grahami	Present	iii, 7	i, 15–17	iii, 5	i, 8–9	16	61–69	Yes	No	No	Zhao and Zhang 2009
S. guishanensis	Present	iii, 7	i, 13–16	iii, 5	i, 7–8	15–16	73–80	Yes	No	No	Li et al. 2003a
S. huaningensis	Present	iii, 7	i, 16	iii, 5	i, 8	16	59–67	Yes	No	Yes	Li et al. 1998
S. huizeensis	Present	iii, 7	i, 15–16	iii, 5	i, 10	18	70–73	Yes	No	No	Cheng et al. 2015
S. xiejiahuai	Absent	iii, 6½	i, 13	iii, 5	i, 7	17	74	No	No	No	Fan et al. 2024
S. bannaensis	Present	iii, 8	i, 9	ii, 5	i, 9	16	47	Yes	Yes	No	Li et al. 2019
S. maculatus	Present	iii, 7	i, 14–15	iii, 5	i, 7-8	16	81–88	Yes	No	No	Zhao and Zhang 2009
S. maitianheensis	Present	iii, 7	i, 14–15	iii, 5	i, 9	18	70–82	Yes	No	Yes	Li 1992
S. malacopterus	Present	iii, 7	i, 14–18	iii, 5	i, 9	15–16	67–81	Yes	No	No	Chu and Cui 1985
S. longifinus	Absent	iii, 7	i, 16	ii, 5	i, 8	17	70–72	No	Yes	Yes	Li et al. 1998
S. macroscalus	Present	iv, 7	i, 15–16	iii, 5	i, 8	NA	70–79	Yes	No	No	Li 1992
S. macrocephalus	Absent	iv, 7	i, 15–17	iii, 5	i, 8	16	72–78	Yes	No	No	Li 1985
S. lateristritus	Present	iv, 7	i, 15–16	iii, 5	i, 8	17	75–91	Yes	No	No	Li 1992
S. purpureus	Present	iv, 6–7	i, 16	iii, 5	i, 8	NA	63–67	Yes	No	No	Li 1985
S. qiubeiensis	Present	iii, 7	i, 14–17	iii, 5	i, 8–9	16	67–81	Yes	No	No	Li et al. 2002b
S. qujingensis	Absent	iii, 7	i, 16	iii, 5	i, 8	16	70–79	Yes	No	No	Li et al. 2002c
S. robustus	Present	iv,7	i,13	iii, 5	i, 6	16	72	No	No	No	Chen et al. 1988b; This study
S. wumengshanensis	Present	iii, 7	16	ii, 5	i, 8	16	67–76	Yes	Yes	Yes	Li et al. 2003a
S. xichouensis	Present	iii, 6–7	i, 14–16	iii, 5	i, 8–9	NA	74–88	Yes	Yes	No	Pan et al. 2013
S. tingi	Present	iv, 7	i, 14–16	iii, 5	i, 6–8	16	62–73	Yes	No	No	Zhao and Zhang 2009
S. yangzongensis	Absent	iii, 7	i, 16	iii, 5	i, 9	16	71–81	Yes	No	No	Zhao and Zhang 2009
S. yimenensis	Present	iii, 7	i, 14–15	ii, 5	i, 8	16-17	70–79	Yes	No	No	Li et al. 2005
S. oxycephalus	Present	iii, 7	i, 15–16	iii, 5	i, 8–9	17	62–75	No	No	No	Li 1985; Zhao and Zhang 2009
S. wenshanensis		1	i, 13–15	ii, 5		14–15	67–72	Yes	No	Yes	Yang et al. 2018
S. gracilis	Absent	NA –	NA	NA 	NA . – •	NA	NA	Yes	NA	NA	Li and Li 2014
S. pingshanensis	Absent	iii, 7	i, 13–15	ii, 5	i, 7–8	16	75–78	Yes	Yes	No	Wu et al. 2018
S. luolouensis	Present	iii, 7 □ –	i, 13–14	iii, 5	i, 7–8	16–17	40–49	Yes	Yes	Yes	Lan et al. 2013
S. wui	Absent	iii, 7	i, 14–15	ii, 5	i, 7–8	14–15	79–81	Yes	No	No	Li and An 2013



**Figure 4.** View of adult holotype GZNU20190508001 of *Sinocyclocheilus xingrenensis* sp. nov. in preservative. **A.** Lateral view; **B.** Dosal view; **C.** Ventral view of the head; **D.** Dosal view of the head.

distance between the origin and the anus, tips not reaching the caudal fin base. Caudal peduncle length 18–23% of SL, depth 47–67% of body depth. Caudal fin forked, upper lobe equal in length to the lower one, tips truncated.

Body scaleless. Lateral line complete and slightly curved, with pores 65–77, originating from upper margin of operculum and extending to end of caudal peduncle. With about 30 to 58 irregular black markings scattered above the lateral line, almost in a straight line, and a large black spot in the center of the end of the caudal peduncle.

Coloration and variation. In life, the body is golden yellow overall, with white pectoral fins and a slightly translucent white dorsal fin; the gills are blackish, with numerous black markings on the back and a relatively large black spot at the caudal fin base (Fig. 5). In 7% formalin solution, the specimens are dark gray above the lateral line and white below the lateral line, with each fin yellowish white (Fig. 4). Most specimens are consistent in their morphological characteristics, except for specimen GZNU20190508004, where the lateral line separates

backward into two from the upper pectoral fin and converges at the base of the caudal fin.

**Distribution.** Sinocyclocheilus xingrenensis sp. nov. is found only in caves near Xingren City, Guizhou, China, including the type locality and from Gaowu Village, Xiashan Town, Xingren City. Much ecological information about the new species is currently unknown. The discovery site belongs to the Beipanjiang River basin.

Etymology. The specific epithet "xingrenensis" refers to the type locality of the new species: Xingren City, Guizhou Province, China. We propose the common English name "Xingren Golden-lined Fish" and the Chinese name "Xīng Rén Jīn Xiàn Bā (兴仁金线鲃)".

#### Discussion

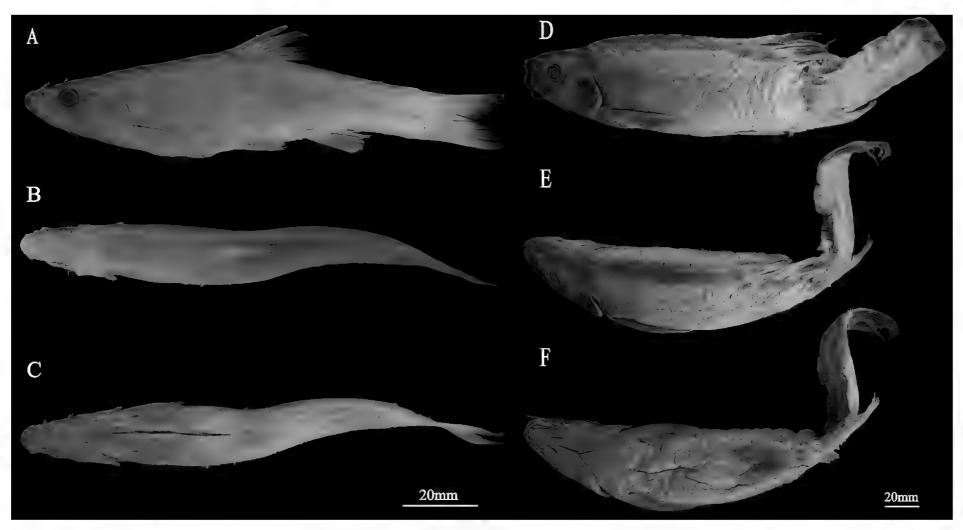
This new species, named *Sinocyclocheilus gaowuensis*, was described by Liu (2018a) on the basis of single-numbered specimens and genetic information. However, this work

**Table 4.** Morphological characterization and statistical analysis of new species and *S. angustiporus*. Abbreviations: *Sx*, *S. xingrenensis* sp. nov., and *Sa*, *S. angustiporus*.

Character	S.	xingrenensis sp.	nov.	S. angu	P-value from ANOVA	
	Holotype	Range	Mean ± SD	Range	Mean ± SD	Sx vs. Sa
Dorsal fin rays	iii, 7	iii, 6–7	NA	iv, 7	NA	NA
Pectoral fin rays	i, 15	i, 12-16	NA	i, 14	NA	NA
Pelvic fin rays	i, 8	i, 8	NA	i, 8	NA	NA
Anal fin rays	iii, 5	iii, 5	NA	iii, 5	NA	NA
Caudal fin rays	15	15–18	NA	16–17	NA	NA
Lateral line pores	65	65–76	NA	NA	NA	NA
Total length	149.4	99.0-149.4	$114.5 \pm 17.2$	55.40-128.08	$73.9 \pm 30.4$	0.014
Standard length	123.5	79.7–123.5	$92.6 \pm 15.0$	43.03-104.35	$59.2 \pm 25.5$	0.017
Body depth	24.6	16.7-24.6	$19.3 \pm 2.5$	10.24-24.59	$14.6 \pm 5.7$	0.077
Head length	31.9	22.8-31.9	$25.0 \pm 3.1$	12.08-27.19	$15.8 \pm 6.4$	0.008
Head depth	21.5	14.3-21.5	$15.9 \pm 2.6$	7.36-19.46	$10.3 \pm 5.1$	0.032
Head width	16.2	11.5-16.2	$13.3 \pm 1.5$	5.05-12.54	$7.4 \pm 3.0$	0.001
Inter-Pre-Nasal Distance	5.1	4.8-6.2	$5.4 \pm 0.5$	1.84-4.59	$2.5 \pm 1.2$	0.000
Posterior Nasal Distance	6.9	4.2-6.9	$5.2 \pm 0.9$	2.23-5.10	$3.1 \pm 1.2$	0.005
Upper jaw length	9.4	5.0-9.4	$6.2 \pm 1.6$	2.76-6.56	$3.8 \pm 1.6$	0.029
Lower jaw length	7.9	4.8–7.9	$5.6 \pm 1.2$	2.36-6.35	$3.5 \pm 1.6$	0.028
Mouth width	8.2	6.2-8.2	$6.9 \pm 0.6$	2.50-5.29	$3.3 \pm 1.1$	0.000
Eye diameter	5.0	4.1-5.0	$4.7 \pm 0.3$	3.09-5.32	$3.7 \pm 0.9$	0.020
Interorbital distance	9.0	7.5–9.0	$8.3 \pm 0.5$	3.32-7.61	$4.7 \pm 1.7$	0.000
Dorsal-fin base length	17.4	10.7-17.4	$12.5 \pm 2.3$	6.07-15.22	$8.3 \pm 3.9$	0.039
Dorsal-fin length	24.7	15.3-24.7	$19.9 \pm 3.1$	10.10-22.62	$13.1 \pm 5.4$	0.019
Pectoral-fin length	23.2	14.1-23.2	$17.9 \pm 2.9$	8.35-17.54	$10.5 \pm 4.0$	0.004
Pectoral-fin base length	4.1	2.8-4.1	$3.4 \pm 0.4$	1.97-3.78	$2.5 \pm 0.7$	0.023
Prepectoral length	32.9	21.7-32.9	$25.0 \pm 3.7$	12.67-28.45	$16.5 \pm 6.7$	0.018
Pelvic-fin length	18.5	11.3-18.5	$14.6 \pm 2.4$	6.73-14.53	$8.8 \pm 3.3$	0.006
Pelvic-fin base length	7.7	4.0-7.7	$4.9 \pm 1.3$	1.62-4.37	$2.4 \pm 1.1$	0.005
Prepelvic length	59.1	39.2-59.1	$45.6 \pm 6.6$	21.90-55.21	$29.9 \pm 14.2$	0.027
Anal-fin length	17.8	12.4–17.8	$14.6 \pm 1.9$	7.84-13.40	$9.5 \pm 2.3$	0.002
Anal-fin base length	11.1	6.4–11.1	$8.0 \pm 1.5$	3.92-9.70	$5.4 \pm 2.4$	0.044
Preanal length	82.7	55.7-82.7	$62.5 \pm 9.5$	30.68-75.53	41.8 ± 18.9	0.031
Caudal peduncle length	22.3	16.4-22.3	$18.5 \pm 2.2$	9.01-18.11	$12.3 \pm 3.4$	0.003
Caudal peduncle depth	13.1	8.7–13.1	$10.5 \pm 1.8$	4.90-12.49	$7.3 \pm 3.0$	0.040
Maxillary barbel length	10.4	7.7–13.5	$10.3 \pm 2.1$	3.20-8.05	$4.5 \pm 2.0$	0.001
Rostral barbel length	13.2	9.8–13.2	$11.5 \pm 1.4$	3.17–10.07	$5.0 \pm 2.9$	0.000



Figure 5. Live paratype of Sinocyclocheilus xingrenensis sp. nov.



**Figure 6.** Holotype specimens from *Sinocyclocheilus angustiporus* (IHB12209016-81X2001, **A–C**) and *Sinocyclocheilus robustus* (IHB12209038-8001091, **D–F**). **A, D.** Lateral view; **B, E.** Dosal view; and **C, F.** Ventral view.



Figure 7. Habitat of Sinocyclocheilus xingrenensis sp. nov.

was not widely accepted because it was an unpublished master's thesis, and the sequences were not deposited online. In this study, we formally describe *Sinocyclocheilus xingrenensis* sp. nov. based on multiple specimens and genetic data. The new species is morphologically (see above) and genetically distinguishable from other species of the genus *Sinocyclocheilus*.

Multiple lines of evidence suggest that the new species did not exhibit strict cave adaptations as young burrowers. Compared to strictly cave-dwelling species, such as S. longicornus (eye diameter 0.0–6.1% of HL) (Xu et al. 2023) and S. sanxiaensis (eye diameter 7.3% of SL) (Jing et al. 2019), the new species exhibit significantly larger eyes (eye diameter 16–21% of HL). This is related to the habitat, where the new species can be connected to surface streams through cave windows (Fig. 7), and their life rhythms may be closely related to photoperiods. Within the genus Sinocyclocheilus, we also mapped the distribution of scales in the phylogenetic tree, which showed that scale loss occurs in only a few species. Using the time of divergence of S. xiejiahuai as a reference (Fan et al. 2024), the loss of these scales occurred centrally after the Pleistocene ( $\sim$ 2.58 million years ago). This suggests that scale degeneration may not be very ancient, ranging from early to late Pleistocene (Policarpo et al. 2021). This is similar to the degeneration or loss of eyes of species within the genus Sinocyclocheilus during the Pleistocene (Mao et al. 2021). This further suggests that most cave fishes may not have lived in cave ecosystems for more than a few million years. Thus, together with the eyes and scales, we hypothesize that the new species may be undergoing a gradual evolutionary process towards caves.

Although the population of this new species is not extremely small, its habitat is located in the center of the village, making it more susceptible to anthropogenic disturbance. Moreover, with the potential for future urbanization in the village, there is a significant risk of habitat degradation and destruction in the near future. On 5 February 2021, the Chinese government designated all species of *Sinocyclocheilus* endemic to China as second-class national protected animals (National Forestry and Grassland Administration & National Park Administration, 2021). Consequently, this species will also require appropriate conservation measures to mitigate the potential threats posed by future anthropogenic disturbances.

### Author contributions

Jiang Zhou and Ming-Yuan Xiao conceived and designed the research; Ming-Yuan Xiao, Jia-Jia Wang, Tao Luo, and Jia-Jun Zhou conducted field surveys and collected samples; Tao Luo and Jia-Jia Wang performed molecular work; Ming-Yuan Xiao, Jia-Jia Wang, and Ning Xiao processed the English language of the manuscript; Jiang Zhou provided financial support. All authors read and approved the final version of the manuscript.

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# Appendix 1

For specimen information examined by the *S. tingi* group (18 species).

- **Sinocyclocheilus aluensis** (N = 2): China: Yunnan Province: Luxi County: Yipu Village (type locality). Currently these specimens are stored by Hong-Fu Yang at the fisheries workstation in Qubei County, Yunnan Province, China.
- Sinocyclocheilus angustiporus (N = 5): China: Guizhou Province: Xinyi City (type locality): IHB12209016-81X2001, IHB12209016-81X2004, IHB12209016-81X2009, IHB12209016-81X2010, IHB12209016-81X2011. These specimens are stored at the Institute of Hydrobiology, Chinese Academy of Sciences, China.
- **Sinocyclocheilus robustus** (N = 1): China: Guizhou Province: Xinyi City (type locality): IHB12209038-8001091. Currently preserved in the Institute of Hydrobiology, Chinese Academy of Sciences, China.
- *Sinocyclocheilus anophthalmus* (N = 1): China: Yunnan Province: Yiliang County: Jiuxiang Township (type locality): KIZ1986003899. Currently preserved in the Kunming Institute of Zoology, Chinese Academy of Sciences, China.
- *Sinocyclocheilus grahami* (N = 1): China: Yunnan Province: Kunming City: Dianchi Lake (type locality): KIZ2007003941. Currently preserved in the Kunming Institute of Zoology, Chinese Academy of Sciences, China.
- Sinocyclocheilus huaningensis (N = 1): China: Yunnan Province: Huaning County (type locality): GZNU20221108001. Currently preserved in Guizhou Normal University, Guiyang City, Guizhou Province, China.
- Sinocyclocheilus huizeensis (N=2): China: Yunnan Province: Huize County: Wuxing Village (type locality): GZNU20160313001. Currently preserved in Guizhou Normal University, Guiyang City, Guizhou Province, China; China: Yunnan Province: Huize County: Dalong Spring (type locality): KIZ2013001246 (holotype). Currently preserved in the Kunming Institute of Zoology, Chinese Academy of Sciences, China.

- Sinocyclocheilus lateristriatus (N = 6): China: Yunnan Province: Luliang County (type locality): GZNU20230825003–0825008. Currently preserved in Guizhou Normal University, Guiyang City, Guizhou Province, China.
- Sinocyclocheilus macrocephalus (N = 1): China: Yunnan Province: Luliang County (type locality): GZNU20230501004. Currently preserved in Guizhou Normal University, Guiyang City, Guizhou Province, China.
- Sinocyclocheilus maitianheensis (N = 3): China: Yunnan Province: Yiliangjiu Village (type locality): GZNU20230824001, GZNU20240105001–0105002. Currently preserved in Guizhou Normal University, Guiyang City, Guizhou Province, China.
- Sinocyclocheilus malacopterus (N = 5): China: Yunnan Province: Luoping County: KIZ1980001282. Currently preserved in the Kunming Institute of Zoology, Chinese Academy of Sciences, China; China: Yunnan Province: Shizong County: Wulong Village (type locality): FWOQB 20180916001–0916004, collected by Hongfu Yang on 20 March 2018. Currently these specimens are stored by Hong-Fu Yang at the fisheries workstation in Qubei County, Yunnan Province, China.
- Sinocyclocheilus oxycephalus (N = 3): China: Yunnan Province: Shilin County (type locality): GZNU20230501001–0501003. Currently preserved in Guizhou Normal University, Guiyang City, Guizhou Province, China.
- Sinocyclocheilus purpureus (N = 1): China: Yunnan Province: Kaiyuan City: Zhongheying Town: Qiaotou Village (type locality): GZNU20200912001. Currently preserved in Guizhou Normal University, Guiyang City, Guizhou Province, China.
- *Sinocyclocheilus qiubeiensis* (N = 1): China: Yunnan Province: Qiubei County (type locality): GZNU20210728001. Currently preserved in Guizhou Normal University, Guiyang City, Guizhou Province, China.
- **Sinocyclocheilus qujingensis** (N = 2): China: Yunnan Province: Qujing County (type locality): GZNU20230825001–0825002. Currently preserved in Guizhou Normal University, Guiyang City, Guizhou Province, China.

Sinocyclocheilus tingi (N = 3): China: Yunnan Province: Yuxi City: Jiangchuan District (type locality): GZNU20230404001–0404003. Currently preserved in Guizhou Normal University, Guiyang City, Guizhou Province, China.

**Sinocyclocheilus wenshanensis** (N = 1): China: Yunnan Province: Wenshan City: Dehou Town (type locality): GZNU20200625001. Currently preserved in Guizhou Normal University, Guiyang City, Guizhou Province, China.

*Sinocyclocheilus xichouensis* (N = 1): China: Yunnan Province: Xichou County: Xingjie Town (type locality): GZNU20210730001. Currently preserved in Guizhou Normal University, Guiyang City, Guizhou Province, China.

**Sinocyclocheilus xiejiahuai** (N = 1): China: Guizhou Province: Panzhou City: Hongguo Town (type locality): GZNU20230304001. Currently preserved in Guizhou Normal University, Guiyang City, Guizhou Province, China.

## Supplementary material 1

# Uncorrected *p*-distance (%) between 24 species of the genus *Sinocyclocheilus* based on mitochondrial Cyt *b*.

Authors: Ming-Yuan Xiao, Jia-Jia Wang, Tao Luo, Jia-Jun Zhou, Ning Xiao, Jiang Zhou

Data type: xls

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Link: https://doi.org/10.3897/zse.101.141444.suppl1

## Supplementary material 2

# Uncorrected *p*-distance (%) between 22 species of the genus *Sinocyclocheilus* based on mitochondrial ND4.

Authors: Ming-Yuan Xiao, Jia-Jia Wang, Tao Luo, Jia-Jun Zhou, Ning Xiao, Jiang Zhou

Data type: xls

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## Supplementary material 3

PCA loadings of the five principal components extracted from 29 morphometric data for *S. robustus*, *Sinocyclocheilus xingrenensis* sp. nov., and *S. angustiporus* 

Authors: Ming-Yuan Xiao, Jia-Jia Wang, Tao Luo, Jia-Jun Zhou, Ning Xiao, Jiang Zhou

Data type: xlsx

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